providing information about the structure and dynamics of several proteins, the details of its characterization is lacking. The goal of this research is to gain insights into the behavior of the BSL that has been so useful in providing details to characterize a variety of protein systems. Fmoc solid phase peptide synthesis (SPPS) has been used to generate a series of double cysteine mutants of the 23 amino acid α-helical membrane peptide, AChR M2delta at i and i+4 residue positions. BSLs bearing peptides were incorporated into 1,2-Dimyristoyl-sn-Glycero-3-Phosphocholine (DMPC) bicelles and multilamellar vesicles. The spectra were collected at different temperatures utilizing continuous wave (CW)-EPR spectroscopy to examine the dynamics of the BSL within a membrane. The EPR spectral lineshape analysis will be conducted to obtain the static and dynamic EPR parameters. This study will provide more accurate insights into the behavior of BSL in order to provide the optimal conditions for other studies that may utilize this promising spin label.

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EPR Spectroscopic Study of the Voltage-Sensor Domain (VSD) of the Human KCNQ1 Potassium Ion Channel

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KCNQ1 is a voltage-gated potassium channel modulated by members of the KCNE protein family. KCNQ1 is essential to both the cardiac action potential that mediates heartbeat and K^+ homeostasis in the inner ear. KCNQ1 dysfunction has been linked to multiple diseases, including various cardiac arrhythmias, congenital deafness, and type II diabetes mellitus. Human KCNQ1 is a 676-residue protein consisting of 100-residue N-terminal cytosolic domain, followed by an ~260-residue channel domain containing six transmembrane (TMD) helices, and a 300-residue cytosolic C-terminus. The first four TMD helices (S1-S4) form the voltage-sensor domain (VSD) that is linked to the pore domain (helices S5 and S6). Roughly 40% of the >200 reported disease-related mutations in the KCNQ1 gene result in amino acid substitutions in the VSD, making structural and dynamic studies of this domain important in unraveling molecular mechanisms in human pathophysiology. Site-directed spin labeling EPR is a very powerful structural biology technique to study the structural and conformational dynamics of membrane proteins in membrane environment. In this study, we have successfully expressed the Cysteine substituted Q1-VSD in E. Coli, purified into detergent micelles, labeled with MTSL spin labels, and collected CW-EPR spectra. We are developing several advanced EPR spectroscopic techniques (such as double electron-electron resonance (DEER) and electron spin echo envelope modulation (ESEEM)) for probing the structural and dynamic properties of Q1-VSD in a lipid bilayer environment. This study will provide important mechanistic information on the Q1-VSD channel upon binding with KCNE1.

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Combining Single Crystal Electron Paramagnetic Resonance and X-Ray Crystallography to Study the Orientation and Dynamics of MTSSL Spin Labels in T4 Lysozyme

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The line shape of cw electron paramagnetic resonance (EPR) spectra of spin labeled proteins has been used extensively to analyze local dynamics, backbone fluctuations and structural rearrangements of these systems. The commonly used paramagnetic spin label MTSSL, a methanethiosulfonate derivative, attached to the protein via covalent disulfide coupling on a designated cysteine residue serves as a reporter of these properties. Much effort has been made to extract structural and dynamic information as encoded in the observed line shapes.

In this study we use single crystals of MTSSL labeled T4 lysozyme as a prototype example of an α-helical protein to investigate the structure and dynamics of the paramagnetic side chain in detail by a combination of X-ray crystallography and EPR spectroscopy. X-ray diffraction is used to determine the orientation of the unit cell with respect to the EPR sample tube, which in turn allows to determine the orientation of the spin labels for every EPR spectrum of an angular dependent series given that the structure and orientation of the protein in the unit cell is known. To this end high-resolution crystal structures (0.9 - 1.3 Å) of spin labeled T4 lysozyme were taken at 100 K and are compared to data deposited in the protein data bank.

On this poster we will discuss the angular dependent line shape of the EPR spectra taken at room temperature with respect to the orientation of the spin label as deduced from X-ray crystallography at 100 K. Particular emphasize will be on the ability of currently available models to describe the observed dynamics of the spin label and the implications of this for the line shape analysis of disordered samples.

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Uncertainty Quantification in Deer Spectroscopy using Bayesian Statistical Inversion Methods

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Double electron-electron resonance (DEER) spectroscopy is a powerful electron paramagnetic resonance experiment that provides 2-8nm distance measurements between spin-labels in systems varying from small, soluble proteins, to large, membrane-bound complexes. A key strength of DEER is that it reports a distance distribution corresponding to conformational ensembles in frozen solutions. However, in practice its usable information is often limited to the dominant spin-spin distance. Peak widths and shapes, which describe conformational heterogeneity, are highly sensitive to time-domain noise, regularization parameters, and spin-label conformations. A robust method for quantifying uncertainty in DEER distributions does not currently exist, complicating interpretation. Here we demonstrate the use of Bayesian statistical inversion methods to generate conditional covariance estimates and Bayesian credibility sets in DEER data analysis. To decouple true uncertainty from spin-label conformations, we attached spin labels featuring varying flexibilities to maltose binding protein (MBP) and measured using DEER under varying conditions. By determining the contributions to DEER data from both the spin-label and uncertainty, we have taken an important step towards robust assignment of distance distribution features to protein conformations.

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Characterization of Calmodulin Binding to the Ryanodine Receptor by Solution and Solid-State NMR

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Calmodulin (CaM) is a 16.7 kDa, Ca^{2+}-sensing protein that regulates a number of cellular targets based on the amount of Ca^{2+} available in a cell. One of CaM's cellular targets is the ryanodine receptor (RyR), a tetrameric Ca^{2+} channel involved in Ca^{2+} release from the sarcoplasmic reticulum (SR) in myocytes. CaM's regulation of the RyR has been shown to be disrupted due to both oxidative modification and naturally occurring mutations in CaM, however the structural aspects of CaM's binding to the full RyR, and how this binding is disrupted in disease states, has not been completely characterized.

By applying a combination of solution and solid-state NMR techniques we are able to characterize the structure and dynamics of CaM's binding interaction with the RyR on a residue-specific basis in a biologically relevant environment. Solution-state NMR experiments provide an assessment of the changes in fast timescale dynamics with exposure to the SR, while magic angle spinning (MAS) solid-state NMR in both extracted SR and reconstituted lipids characterize conformational and topological changes with CaM in the presence of the RyR. Our results indicate that CaM does exhibit changes in chemical shift and dynamics in the presence of the RyR, and that oxidation and disease mutations of CaM exhibit structural and dynamics changes compared to WT CaM. These results are being applied toward the determination of a structural model for CaM bound to the RyR, and structural models of how this interaction is altered in the development of disease states.

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Confined Space, Structural Biology, Biophysics and Drug Discovery

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High-resolution multi-dimensional solution NMR is unique as a biophysical and biochemical tool in its ability to examine both the structure and dynamics of macromolecules at atomic resolution. Conventional non-TROSY/deuteration solution NMR approaches, however, are largely limited to examinations of relatively small (< 25 kDa) molecules, mostly due to the